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BIOLOGICAL BULLETIN

SOME REMARKS ON THE GASTRULATION OF DESMOGNATHUS FUSCA.

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For the last three or four years I have been trying to secure material to complete observations made some time ago on the gastrulation of *Desmognathus*. There are many difficulties in the way of obtaining the right stages for this study, for the eggs are without pigment and enclosed in very tough membranes. Added to the difficulties of selection and orientation, is the fact that the eggs are large and not easily sectioned.

As mentioned in an earlier paper,[1] the eggs of this species are holoblastic although approaching the meroblastic type of seg-

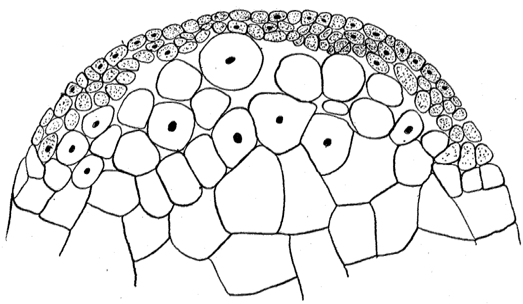

FIG. 1. Upper half of a late morula stage of *D. fusca*. The dotted cells are those with small yolk granules. ×25.

mentation. A moderate-sized blastula cavity is formed, above it, small cells with small yolk granules and below, large cells with larger granules. This cavity in later stages comes to be more or less filled in with larger or smaller cells which round off from the general mass (Figs. 1 and 2).. To what extent the

[1] W. A. Hilton, "General Features of an Early Development of *Desmognathus fusca*," *Jour. Morph.*, Vol. XX., 1909.

segmentation cavity may in this way normally become filled in, I am at present unable to say, but I have found some specimens every year for the past few years in which the segmentation cavity was totally obliterated. As the cells divide, the small ones of the animal pole gradually extend about the larger yolk cells and, at an early stage, one may recognize at the point of junction of the two masses and in from the surface, some of the small cells which later go to form the mesoblast. These correspond to those recognized by Morgan, King, Ruffini and others.[1] At a stage when the small cells begin to migrate over the larger, there are about three layers of these with small yolk granules at the animal pole. This number later becomes reduced to two and then to one, similar to the single cell layer found by Lange in *Megalobatrachus;*[2] this outer layer or ectoblast becomes more extensive and may be found forming a simple definite covering over a large part of the egg. The other small cells, many if not all of which contain small yolk granules, have by this time migrated considerably, but not so extensively as the ectoblastic. Their exact limits are hard to determine. At about the time the ectoblastic covering at the animal pole has become reduced to one cell, the first indication of gastrulation was found. Fig. 3 is a section of a rather early stage.

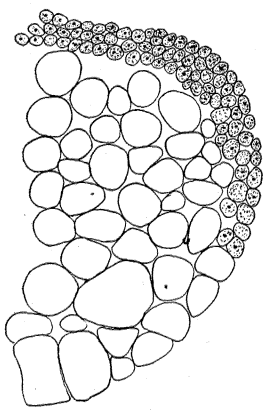

FIG. 2. Portion of a late morula stage of *D. fusca.* ×25.

As mentioned at an earlier time, the external appearance of gastrula stages do not differ from those of other amphibia to any great degree, but some details of development seem peculiar. I now have several series of earlier stages than have been available before and in the earliest of these, I find the gastrula mouth

[1] T. H. Morgan, "The development of the Frog's Egg," 1897. H. D. King, "The Gastrulation of the Egg of *Bufo lentiginosus,*" *Am. Nat.*, Vol. XXXVI., 1902. A. Ruffini, "Contributo alla conoscenza della Ontogenesi degli Amfibi anuri ed urodeli," *Arch. It. Di. Anat. Di. Emb.*, 1907.

[2] D. de Lange, Jr., "Die Keimblätterbildung des *Megalobatrachus maximus* Schlegel," *Anat. Hefte*, Bd. 32, H. 3, 1907.

well down on the yolk mass and appearing as a cleft between the small cells which have grown down about the larger ones. This early cleft in all of my specmiens appears to be not entirely formed by invagination. In later stages, however, there is good evidence of ingrowth especially from the small cells of the upper lip. In a number of specimens of this stage, the rounded yolk cells which were in or near the segmentation cavity are more or less artificially crowded together, probably due to the loss of fluid between them in the passage through the alcohols, so that no clear idea was obtained of their positions. It is probable that about the time of gastrulation there is a considerable change of position of cells at the animal pole of the egg, the cells may

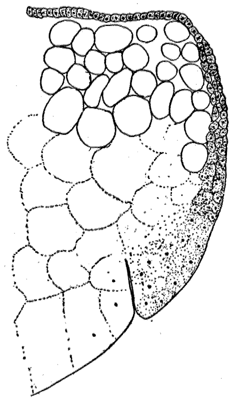

FIG. 3. The beginning of gastrulation of D. *fusca* indicated by a cleft. Specimen shrunken somewhat in the alcohols. ×25.

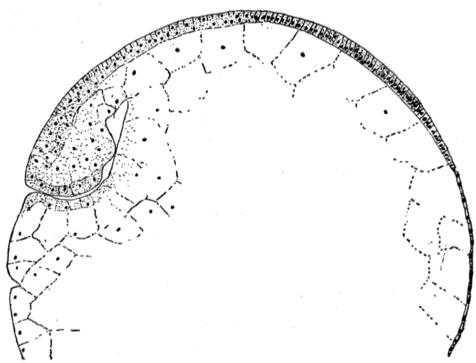

FIG. 4. Section through a later stage of gastrulation. Small cells have penetrated from the surface and a cleft between the cells is added. ×25.

have a looser arrangement, there may even be considerable areas between them occupied by fluid, evidence of which may be seen in certain living eggs. The loss of this fluid by fixation causes the collapse of the thin roof and the crowding together of the cells at the animal pole (Fig. 3). However, in many slightly later stages including both earlier specimens and those only recently obtained, the segmentation cavity seems to have been largely lost and still later than this, new spaces between the yolk cells make their appearance and communicate directly with

the cavity formed by gastrulation. Fig. 4 is through a specimen of such a later stage where the total cavity, although slight, is clear and definite. The lining of the first part of this is from invaginated cells and some of the original ones which were with small yolk granules on the animal pole may be distinguished as part of the lining of the cavity, the total extent of which is shown in a few sections beyond this level.

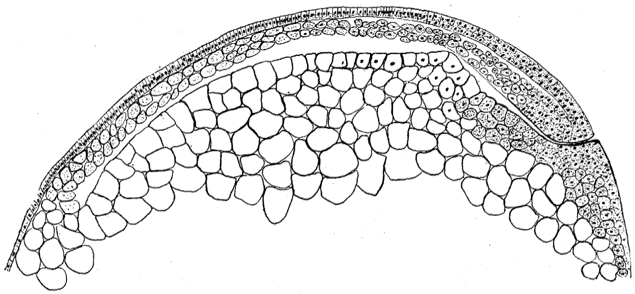

FIG. 5. Longitudinal section through blastopore and archenteron showing dorsal and ventral lip. Small cells with small granules dotted as in the other drawings. ×25.

In later stages the cavity or archenteron may not be very extensive. Cells just under the dorsal ectoblastic ones may be seen to be composed of small yolk granules and beyond the point where invagination is apparent these seem to be well organized and represent a part of the middle germ layer and were probably

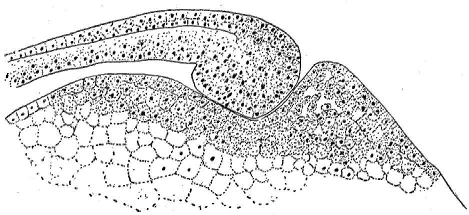

FIG. 6. Longitudinal section through the blastopore of a stage a little later than that of Fig. 5, showing cells which have grown in from the dorsal lip. ×25.

formed from some of the earlier small cells which were traced in their migrations about the yolk mass.

In other specimens the cavity of the archenteron is not very wide, most of the dorsal cells lining it contain small yolk granules,

and these may represent some of the early small cells which have migrated about the yolk. Some of the cells near the opening of the blastopore have grown in dorsally and the ventral lip is also to some degree made up of small cells, some of which may be followed into the floor of the archenteron (Fig. 5).

In later gastrula stages as the enteron becomes more and more marked, there may be noticed a considerable ingrowth of cells, especially from the dorsal lip, in some specimens causing this free portion of the roof to become rolled as Brauer[1] found to be the case in the Gymnophiona (Fig. 6).

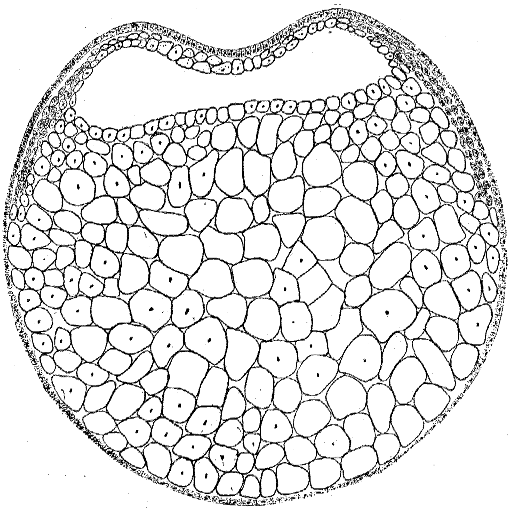

FIG. 7. Cross section through an embryo just before the beginning of the nervous system, showing the lateral extent of the mesoblast. ×25.

At a time when the blastopore is reduced to a very small hole and just before medullary folds are formed, the cavity has come to be large and extensive. Fig. 7 is from a cross section of such a stage. In it the mesoblast has not penetrated very far ventrally especially in the more cephalic region. Later than this, when the neural plate has begun to close, we find that as yet in the head end the mesoblast does not extend much farther but, in the caudal region, it has penetrated between the ectoblast and the

[1] A. Brauer, "Beiträge zur Kenntnis der Entwickelungsgeschichte und der Anatomie der Gymnophionen," *Zool. Jahrb. Anat. Abt.*, Bd. X., 1897.

yolk in all directions from a great mass of cells surrounding the blastopore, extending forward and to a slight extent back of it. This mass of cells, which is continuous cephalad with the nervous system and the mesoblast, is in this more caudal region an undifferentiated group. It is from this that the mesoblast grows out to meet that which has been formed on each side of the notochord (Fig. 8). The place where the mesoblast from the cephalic region and that from this caudal mass meet is often distinguishable because of a difference in the staining character of the cells. In this late stage when the body of the embryo is

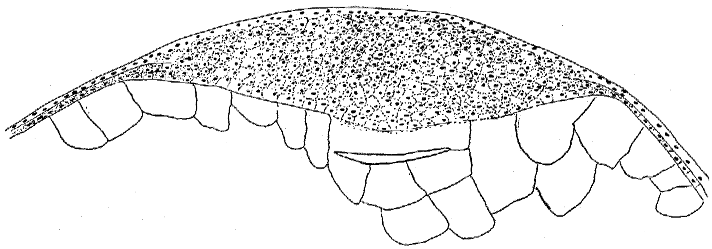

FIG. 8. Cross section through the caudal end of a late medullary plate stage, showing the undifferentiated cell mass, and the small size of the archenteron.

outlined, the cavity of the archenteron is much reduced in size (Fig. 8). This caudal mass is made up from cells on all sides of the blastopore, but especially cephalad of it and evidently represents in large part, the small cells or their progeny which with the narrowing of the blastopore have been brought to the dorsal side of the yolk mass.

In the study of these stages in *Desmognathus*, one is impressed with the fact that fluids within the egg between the blastomeres have more significance than has been generally recognized and it seems possible that this fluid varying with the condition of development, may have considerable importance in the change of form and position of the cells. At times this liquid seems to exert no active influence on the size or condition of the cavities or shapes of the cells, and at others it seems to definitely force the cells into an even layer. For instance in early segmentation stages, those from the yolk mass and other surrounding cells round off toward the blastocœle and tend to fill it in, while at other times, as in late gastrula stages, the edges of the cells

surrounding the archenteron are as smoothly pressed back from the lumen as though some definite substance occupied the large cavity.

The variations from time to time of the cavities within the egg are quite striking. The variations in extent of the segmentation cavity, its reduction and disappearance in some specimens at least, the development especially in the later stages of clefts between cells, the growth in extent of the archenteron and its reduction by the time the nervous system is developed; all of these changes may be dependent upon the large yolk mass and the peculiarities of environment. If this last be a factor of importance in the cavity changes within the eggs, then we might expect to find, as we do, some variation in individuals of the same relative stage of development, for females with eggs or young are found in places that are from wet to almost dry at different seasons or during the same summer.

Some General Conclusions.

1. The small-granuled, small cells of animal pole grow about the larger with greater or less filling in of the segmentation cavity.

2. The cells of the animal pole become reduced to one layer.

3. The first indication of gastrulation appears in the vegetative hemisphere at a region between the small cells which have grown down and the yolk cells.

4. There is an ingrowth of small cells, from the dorsal lip especially.

5. The early segmentation cavity as such probably does not become joined to the invagination.

6. The later archenteron is formed partly by invagination and partly by separation between cells.

7. The cavity of the archenteron is apparently made up of invaginated cells, yolk cells and included under either one or both of these, some of the small cells which have migrated from the region of the animal pole.

8. The small cells at the animal pole form ectoblast.

9. The small cells at the animal pole at least some of which are under the surface cells form mesoblast and probably some entoblast.

10. In later stages of gastrulation there is a marked ingrowth from the dorsal lip of the blastopore.

11. Dorsal and ventral mesoblast become fused in later stages due to the formation of the caudal mass in front of and about the blastopore.

12. The caudal mass furnishes mesoblast in all directions. That formed caudally and laterally grows between the yolk cells and ectoblast. Later, the growing edges of the mesoblast fuse to enclose practically the whole yolk mass.

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